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TECHNICAL MANUSCRIPT 548

ALTERED HOST RANGE BY HOST-CONTROLLED MODIFICATION OF STAPHYLOCOCCUS AUREUS TYPING PHAGE 71

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OCTOBER 1969

DEPARTMENT OF THE ARMY
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ABSTRACT

Phage 71 propagated on Staphylococcus aureus 71 has a calculated efficiency of 6×10^{-9} on S. aureus 100, isolate 6N-15. After propagation on 6N-15, a modified phage is produced that plaques with an efficiency of 1.0 on 6N-15 and 5×10^{-10} on 71.

ALTERED HOST RANGE BY HOST-CONTROLLED MODIFICATION OF *STAPHYLOCOCCUS AUREUS* TYPING PHAGE 71*

During attempts to find a phage with a high efficiency of plating (EOP) on *Staphylococcus aureus* 6N-15, a derivative of *S. aureus* 100,[1] we tested 35 staphylococcal typing phages. None plated on 6N-15 with an EOP higher than 10^{-3}. This report is concerned with a reciprocal host-controlled modification of one of the typing phages, 71, that resulted in a 10^8- to 10^9-fold increase in plating efficiency on 6N-15.

Phages were usually propagated at 37 C in shaken flasks of trypticase soy (TS) broth (BBL) supplemented with 400 μg $CaCl_2$ per ml.[2] Flasks were inoculated with a 10% transfer of a 16-hour culture. Cells were infected at a multiplicity of 0.5 to 1.0 and incubation was usually continued until lysis. Phage 71·6N-15 could also be propagated on TS broth agar + 400 μg $CaCl_2$ per ml (bottom layer 1.5% agar, top layer 0.5% agar).

Phages were assayed by the agar layer technique.[3] For phage 71·6N-15, the bottom layer (25 ml) contained 30 g of TS broth + 400 μg $CaCl_2$ per ml and 15 g of agar (Difco) per liter; the top layer (3 ml) contained 30 g of TS broth + 400 μg $CaCl_2$ per ml and 5 g of agar per liter. Phages 71·71 and 71·6N-15·71 could be assayed on the same medium, but more discrete plaques were obtained when the top layer contained 37 g of brain heart infusion broth (Difco) instead of the TS broth. Indicator cells (5×10^7 per plate) were grown in a modified version of the medium of Chu et al.[1,**] consisting of 4% N-Z-Amine, type NAK (Sheffield), 0.2% yeast extract (Difco), and 0.2% glucose, pH 6.7. Usually, freshly grown 6-hour cells were used, although the cultures could be stored at 4 C for 3 days without appreciable change in plating efficiency.

Table 1 shows the plating efficiencies of restricted and modified phage 71. Phage 71 that had been propagated on *S. aureus* 71 plaqued at an extremely low efficiency on *S. aureus* 6N-15. However, progeny phages (71·6N-15) from a plaque that appeared on 6N-15 following plating of phage 71·71 were found to have undergone reciprocal host-controlled modification.[4] The isolated phage was purified by several single-plaque isolations on 6N-15. Phage 71·6N-15 plaqued with a high efficiency on 6N-15 but its relative efficiency on 71 was only 5×10^{-9}. That this alteration was probably host-controlled and not the result of a mutation can be seen from the results shown in the third line of Table 1. Phage 71·6N-15·71 was isolated from a plaque that appeared on strain 71 after plating 71·6N-15. The EOP of phage 71·6N-15·71 was similar to that of 71·71 on strain 71. The data shown in Table 1 also reveal that the modification altered the EOP on *S. aureus* strain 55. All three restricted and modified phages adsorbed >99% to strains 71 and 6N-15, indicating that the changes in EOP were not the result of tail alterations.

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** M.E. Friedman, personal communication.

TABLE 1. ASSAY OF PHAGES 71·71, 71·6N-15, AND 71·6N-15·71 ON STRAINS OF S. AUREUS

Phage	Plaque-Forming Units/ml on Indicator Bacteria		
	71	6N-15	55
71·71	4.5×10^{10}	2.5×10^{2}	3.2×10^{10}
71·6N-15	6×10^{0}	1.2×10^{10}	$<10^{3}$
71·6N-15·71	9.8×10^{9}	4×10^{1}	1.1×10^{10}

Although host-controlled modification has previously been reported to occur in S. aureus,[5,6] our system is unusual in the magnitude of the restriction and modification observed. For this reason, the phage 71 system should be useful for the investigation of the molecular basis of restriction and modification in S. aureus. We also suggest that it might be possible, through the use of host-controlled modification, to alter the host ranges of the existing typing phages so that they could be used to identify "untypable" strains of S. aureus.

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